

**SEASONAL VARIATION IN ALLOPATRIC POPULATIONS OF  
*ISCHNURA DENTICOLLIS* (BURMEISTER) AND  
*ISCHNURA GEMINA* (KENNEDY)  
(ODONATA: COENAGRIONIDAE)**

JOAN M. LEONG<sup>1</sup> AND JOHN E. HAFERNIK, JR.

Department of Biology, San Francisco State University,  
San Francisco, California 94132

*Abstract.*—We morphometrically evaluate the seasonal variation within two species of damselflies, *Ischnura gemina* (Kennedy) and *Ischnura denticollis* (Burmeister) in allopatry, in order to determine whether the same morphometric characters will be useful species discriminators in an *I. denticollis* and *I. gemina* hybrid zone. Canonical analysis of discriminance reveals that for both species, early emerging individuals are larger with wider heads than those that emerge later. In addition, each species displays other individual patterns of seasonal variation. Both damselfly species are phenetically distinct despite pronounced seasonal variation; this indicates that the morphometric characters used in this study are potentially suitable for use in diagnosis of hybrid zone individuals.

*Key Words.*—Insecta, Odonata, *Ischnura*, seasonal variation, morphometrics

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Seasonal variation within insect species can produce individuals that are remarkably different in a number of morphological characteristics. The full extent of seasonal variation should be quantified, if morphological comparisons are made between two different species that are known or suspected to vary seasonally. Quantification of this variation is essential when the same morphological characteristics that vary seasonally are also potentially useful in the diagnosis of individuals from a hybrid zone. In this study, we morphometrically evaluate the seasonal variation within two species of damselflies, *Ischnura gemina* (Kennedy) and *Ischnura denticollis* (Burmeister) in allopatry, in order to determine whether the same morphometric characters will be useful species discriminators in an *I. denticollis* and *I. gemina* hybrid zone (Leong 1989).

*Ischnura gemina* is an uncommon damselfly restricted to the San Francisco Bay Area, California (Garrison & Hafernik 1981a) and is a candidate for listing as a threatened species. *Ischnura denticollis*, however, is widespread throughout the western United States (Pritchard & Smith 1956). A known hybrid zone extends along the eastern and southeastern areas of the San Francisco Bay region (Leong 1989) and a recently found sympatric population occurs near Suisun Marsh, Solano Co., California (Hafernik, unpublished data). In allopatry, both species are distinguishable by differences in secondary genitalic structure (the abdominal appendages of males and the prothorax of females) (Kennedy 1917, Garrison & Hafernik 1981a), but in the hybrid zone, these differences break down. Consequently, morphometric characters may be more useful species discriminators than traditional genitalic characters in the hybrid zone.

Both species inhabit small pools, creeks or drainage canals throughout their life cycle. Adults of both species exhibit similar color patterns and are sexually di-

<sup>1</sup> Ecology Graduate Group and Department of Entomology, University of California, Davis 95616.

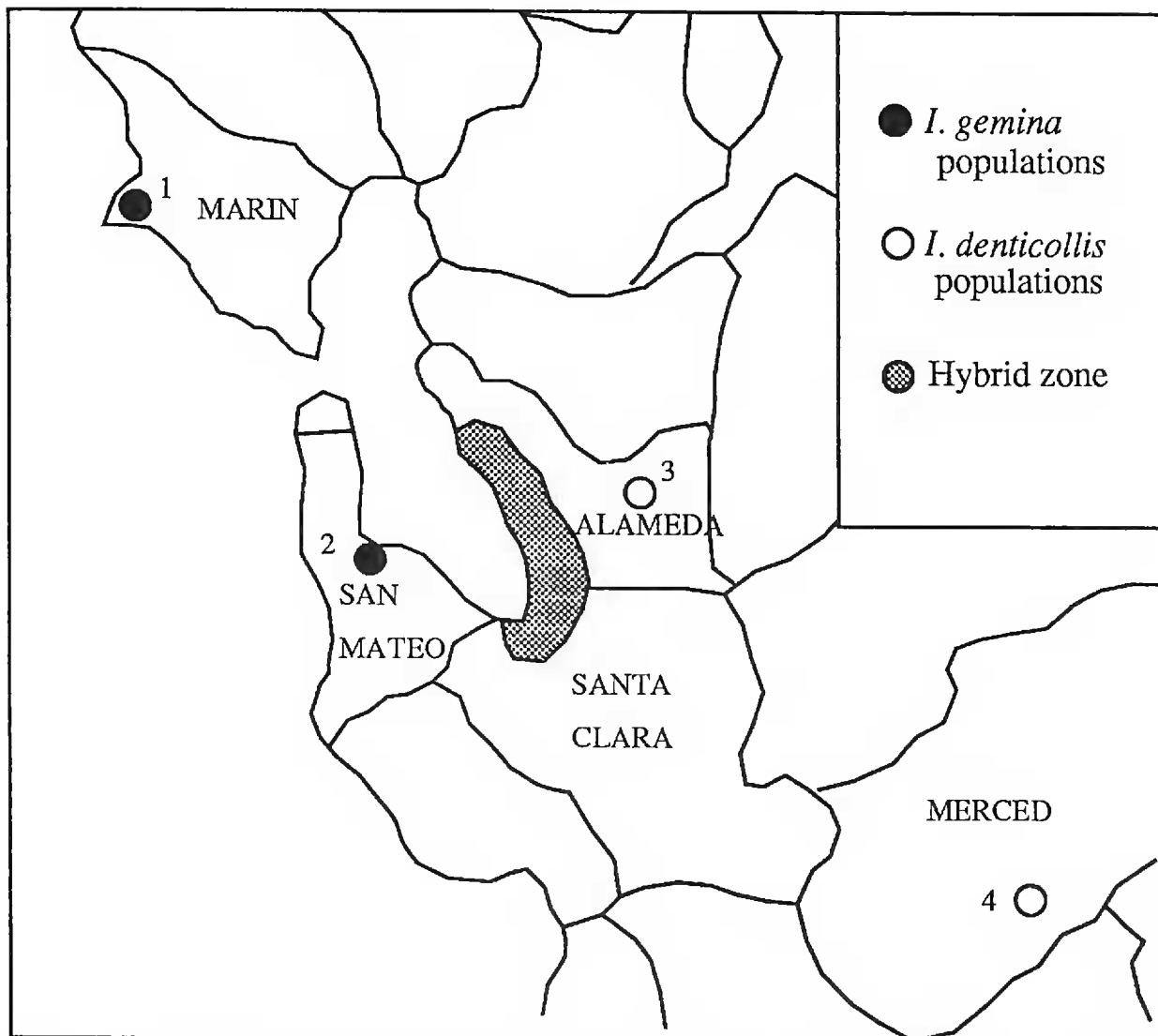


Figure 1. Location of populations sampled. Populations are numbered as follows: 1 = Point Reyes; 2 = Coyote Point; 3 = Livermore; 4 = Los Banos.

morphic; males are more brightly colored than females. Andromorph females, however, present an exception because they possess typical male color patterns. *Ischnura gemina* is slightly larger than *I. denticollis* and in both species, females are generally larger than males. Although some aspects of the life history, population structure (Garrison & Hafernik 1981b), and mating system of *I. gemina* (Hafernik & Garrison 1986; A. Balmy, unpublished data) are well known, the biology of *I. denticollis* is largely unknown.

#### MATERIALS AND METHODS

We sampled two allopatric populations each of *I. gemina* and *I. denticollis* from August 1986 through October 1987 in the San Francisco Bay Area and in the Central Valley of California (Fig. 1 and Table 1). The Coyote Point population of *I. gemina* and the Livermore population of *I. denticollis* were chosen for two reasons: the known allopatric nature of these populations and their proximity to the hybrid zone. Three of the four populations were situated at the vegetated margins of lentic drainage canals and creeks, while the Point Reyes population was located along the edge of a coastal lagoon. We collected samples of 12–16 adult males and 7–17 adult females from each population per sampling period.

The Point Reyes population of *I. gemina* and the Los Banos population of *I. denticollis* were sampled as controls to determine whether or not the Coyote Point and Livermore populations represent typical *I. gemina* and *I. denticollis* popu-

Table 1. Populations of *Ischnura* studied.

Locality	Date	Season	Males	Females	Sample
<i>I. gemina</i>					
Marin Co.: Point Reyes Abbotts Lagoon	8 Sep 1987	Late 1987	14	12	PR
San Mateo Co.: Coyote Point channel along Airport Blvd.	11 Aug 1986	Middle 1986	12	10	CM86
	30 Apr 1987	Early 1987	12	7	CE87
	14 Jul 1987	Middle 1987	14	4	CM87
	3 Aug 1987	Middle 1987	2	4	CM87
<i>I. denticollis</i>					
Alameda Co.: Livermore	8 Aug 1986	Middle 1986	15	1	LM86
Las Positas Creek along Airway Blvd.	7 Sep 1986	Middle 1986	—	9	LM86
	24 Apr 1987	Early 1987	13	8	LE87
	16 Jul 1987	Middle 1987	12	10	LM87
	24 Sep 1987	Late 1987	16	17	LL87
Merced Co.: Los Banos channel near Billy Wright Rd.	4 Oct 1987	Late 1987	14	7	LB

lations. This comparison is important because a population morphologically deviant from the control probably lacks variation that is representative of the species, and would thereby serve as a poor reference group for defining the morphometric characteristics of each species.

We evaluated the importance of seasonal variation within populations by sampling the *I. gemina* Coyote Point and *I. denticollis* Livermore populations an additional two to three times (Table 1). In order to compare effectively temporal variation in these two populations, we collected the pairs of samples synchronously: in August and early September of 1986 and at six to nine week intervals during the 1987 flight season (March through October). Because the life span of *I. gemina* in the field, including maturation time, is as long as four to six weeks (Garrison & Hafernik 1981b; Hafernik & Garrison 1986), we chose a minimum six week sampling interval to insure that individuals that were sampled later in the season did not belong to the cohort sampled previously. Because the lifespan

Table 2. Characters used in multivariate analyses.

Character	Description
Thorax length (TH1)	Length along dorsal median ridge of thorax
Thorax width (TH2)	Greatest width across mesepimera
Thorax depth (TH3)	Distance from posterior corner of metacoxa to dorsal surface of thorax
Head width (H1)	Greatest width between inner margins of eyes along posterior edge of occiput
Head length (H5)	Length from anterior edge of frons to posterior edge of occiput
Wing length (L1)	Length from nodus to outer edge of pterostigma along the coastal margin of the right forewing
Wing width (W1)	Width from nodus to the distal edge of the second antenodal postquadangular crossvein of the right forewing
Wing crossvein count (C1)	Number of postnodal crossveins on the right forewing between R1 and M1 excluding the nodus and the brace vein
Tibia length (T1)	Length from proximal process to distal end of the right protibia
Tibial spine count (C2)	Number of spines along the medial edge of the right protibia

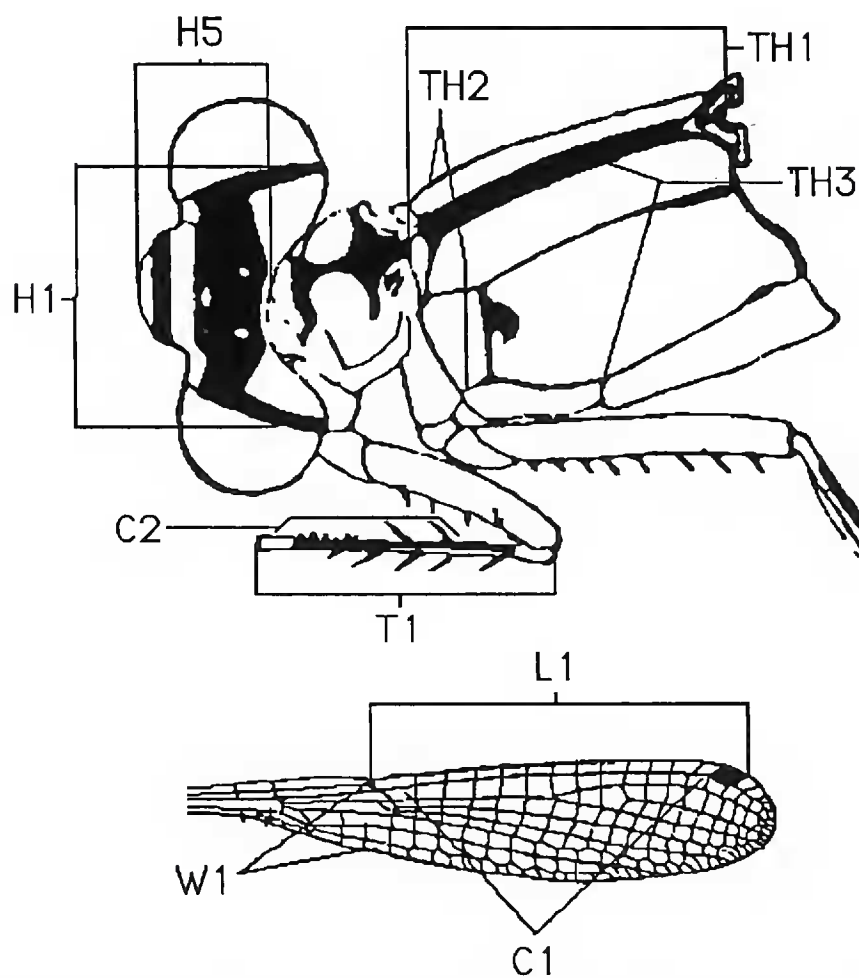


Figure 2. Characters measured. Top figure and bottom figure after Kennedy (1915) and Kennedy (1917), respectively.

of *I. denticollis* is unknown, we assumed that it is similar to *I. gemina*. Sampling dates were categorized as early, middle or late to facilitate comparisons. In two cases, the middle Coyote Point 1987 and the middle Livermore 1986 samples, two samples taken within the six week sampling interval were combined due to small sample sizes of one sex.

Eight continuous characters and two count characters were measured for all individuals (Table 2 and Fig. 2). We did not include body length in the multivariate analyses since it is highly correlated with thorax length ( $r = 0.95$  for females;  $r = 0.91$  for males). We selected these characters to represent the major shape and size attributes of the damselflies. We measured continuous characters to the nearest 0.03 mm using an ocular micrometer and high correlations between repeated measurements (see test-retest reliability, Kachigan 1986) confirmed the reliability of the eight continuous measurements ( $0.96 < r < 0.99$ ;  $P < 0.05$ ;  $n = 66$ ).

A total of 89 females and 124 males were used in canonical analysis of discriminant analysis, a type of discriminant analysis (Pimentel 1979). We analyzed sexes separately to control for sexual size dimorphism. However, we did not analyze andromorph females (those with male coloration) separately because they were not significantly different from heteromorph females in any of the measured characters. We used the Multigroup Discriminant Analysis program in BIOSTAT II (Pimentel & Smith 1986) to analyze the morphometric data. The standardized canonical analysis of discriminant of normal scores model (Pimentel 1979, Pimentel & Smith 1986) was used to interpret the data because both continuous and count characters were included. Each sample listed in Table 1 was entered as a separate group for a total of nine groups.

Multivariate analysis of variance, classification and distance analysis were also

Table 3. Canonical vector coefficients and the percentage of variance explained by each canonical variate.

Character	Females		Males	
	CV 1	CV 2	CV 1	CV 2
TH1	1.379	0.884	-0.277	0.979
TH2	-0.548	0.486	-0.229	-0.95
TH3	0.523	0.780	0.031	-0.363
H1	-0.965	-0.267	1.160	-0.679
H5	-0.312	0.390	0.335	0.607
L1	0.079	-0.14	-0.074	0.764
W1	-0.512	-0.324	0.041	-0.121
C1	-0.438	-0.271	0.490	-0.363
T1	-0.299	-1.373	-0.290	-0.443
C2	-0.326	-0.006	0.259	-0.170
% Variance	75.5%	11.0%	85.7%	6.1%

performed on these groups. Tests of the equality of centroids were checked for significance ( $P < 0.005$ ) before running the canonical analysis of discriminance. Because the results from the distance analysis yielded results similar to the classification of groups, only classification will be discussed. Only the first two canonical variates are discussed because the succeeding axes, in sum, explained less than 14% of the total variation and displayed no discernable patterns.

All specimens are deposited in the Entomology Museum, San Francisco State University, San Francisco, California.

## RESULTS

Canonical variate analysis readily distinguishes *I. denticollis* and *I. gemina*. In both sexes, the samples form discrete clusters along the first canonical variate only (Figs. 3A, 3B), although in males, one *I. gemina* male from Point Reyes is situated at the edge of the *I. denticollis* cluster (Fig. 3B). Characters that are most important in distinguishing between groups along this axis are those with high standardized canonical vector coefficients (Table 3). For females, thorax length, head width and to a lesser extent thorax width and wing width are most important; but for males, head width and the number of wing crossveins are important. The sign of a coefficient indicates the direction in which the character is increasing in magnitude; characters with positive coefficients increase as the axis increases and those with negative coefficients decrease. Thus, the position of both species along with first canonical variate reveals that *I. gemina* females are characterized by wider heads, thoraces and wings relative to thorax length (Fig. 3A). Similarly, *I. gemina* males are characterized by relatively wider heads and greater number of wing crossveins (Fig. 3B). Variation on the first canonical variate accounts for 75.5% of the total variation in females and 85.7% in males (Table 3). Additionally, all individuals were classified to the correct species, except for the *I. gemina* male mentioned above.

Vectors of mixed sign generally indicate that differences in shape rather than size define separation between groups. In both sexes, individuals along the first axis are not ordered by absolute size (Figs. 3A, 3B). Head, thorax and wing width, however, are moderately to highly correlated with thorax length ( $0.76 < r < 0.95$ )

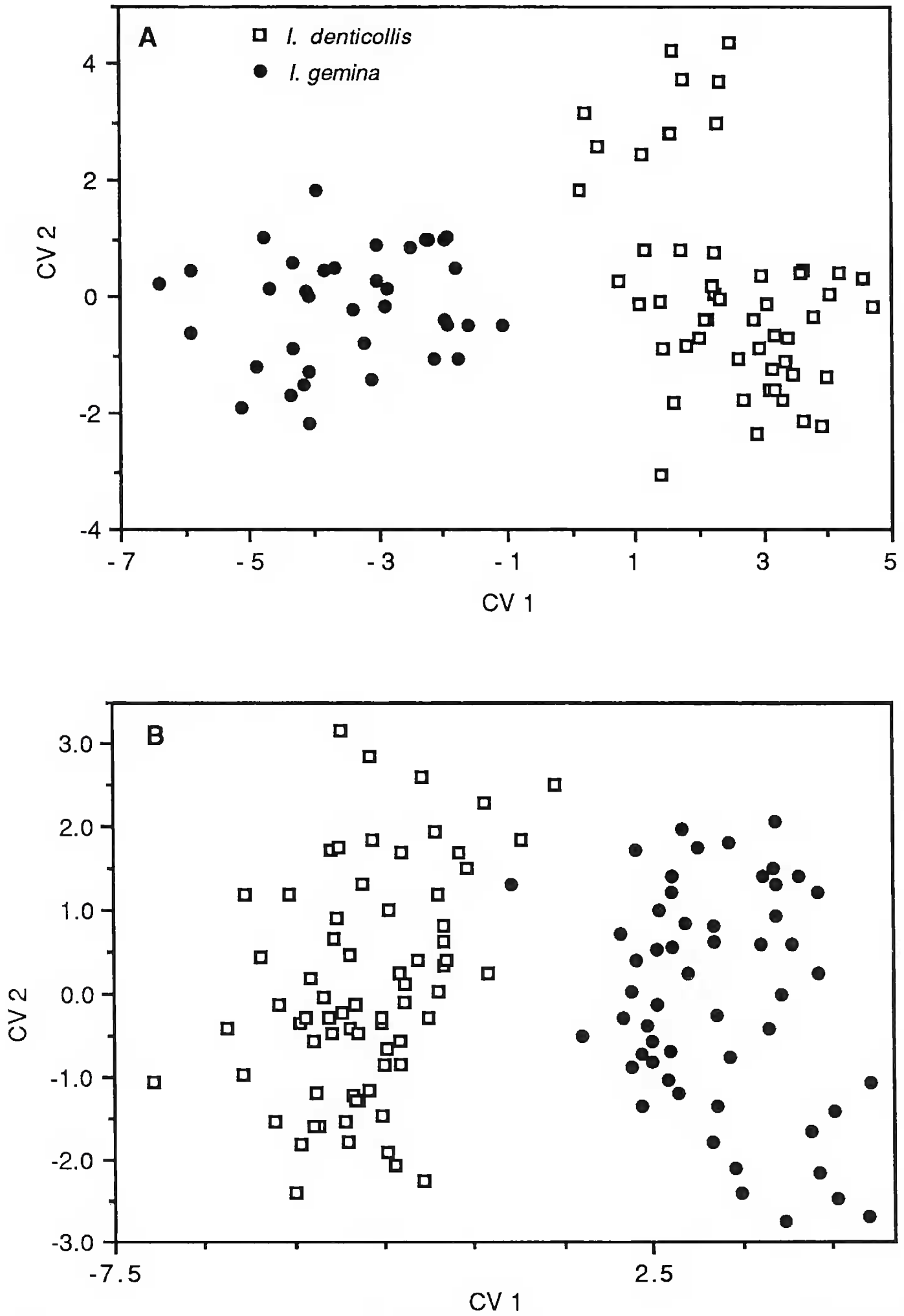


Figure 3. Plot of the first and second canonical variate scores of all individuals. A. Females. B. Males.

which, in turn, is highly correlated with body size. This suggests that the first axis represents a combination of size and shape differences (Table 3). Inspection of the coefficients for the second and third vectors indicates that these axes reflect size and shape differences as well.

The *I. denticollis* Livermore and the *I. gemina* Coyote Point samples exhibit several pronounced patterns of seasonal variation. In *I. gemina* females and males of both species, the same characters that define interspecific differences also distinguish earlier emerging individuals from later ones within the same population. These seasonal changes are evident by the ordering of the Livermore and Coyote Point sample centroids and 95% confidence ellipses along the first axis (Figs. 4A, 4B). In particular, this axis distinguishes early emerging *I. gemina* females (Fig. 4A). All earlier emerging individuals possess proportionately wider heads; in addition, *I. gemina* females have proportionately wider thoraces and wings but earlier emerging males have a greater number of wing crossveins (Table 3). In *I. denticollis* females, however, the early, middle and late Livermore centroids are not clearly differentiated on the first canonical variate.

The second canonical variate also represents a pattern of seasonal variation that distinguishes earlier emerging individuals from later ones, but one that is more distinct than the previous pattern. The positions of the Livermore samples along the second canonical variate (Fig. 4A) reveal that earlier emerging *I. denticollis* females are differentiated from later seasonal samples by longer, wider and deeper thoraces and relatively shorter tibiae (Table 3). However, females from the *I. gemina* Coyote Point samples do not show this second seasonal pattern, and characters which discriminate early emerging *I. gemina* females from later ones are clearly different from those for *I. denticollis* females. In males, the positions of the Livermore and Coyote Point samples on the second axis indicate that the *I. denticollis* and *I. gemina* populations exhibit reversed seasonal patterns (Fig. 4B). Earlier emerging *I. gemina* individuals tend to possess proportionately shorter thoraces, heads and wings while earlier emerging *I. denticollis* males have proportionately longer thoraces, heads and wings (Table 3). The seasonal pattern represented by the second canonical variate explains 11.0% and 6.1% of the total variation in females and males respectively.

The classification of individuals to the correct seasonal population sample provides another measure of the seasonal differentiation within the *I. denticollis* Livermore and *I. gemina* Coyote Point populations. Most females were classified correctly (84%) as were males (70%). Classification of females suggests that the *I. gemina* Coyote Point samples are more differentiated seasonally than the *I. denticollis* Livermore samples. Of the Coyote Point samples, only two individuals from the CE87 sample were misclassified whereas three of the four Livermore samples had two individuals misclassified each. Only one female from the LE87 sample was misclassified, and *I. denticollis* females from other samples were never misclassified as LE87.

Classification of males reveals that they are less seasonally differentiated than females. The Coyote Point samples had four to five misclassifications each except for CE87, which had only one misclassification. Similarly, the Livermore samples had four to seven misclassifications each except for LE87, which had only one misclassification.

The morphometric characterization of the *I. gemina* Point Reyes and *I. den-*

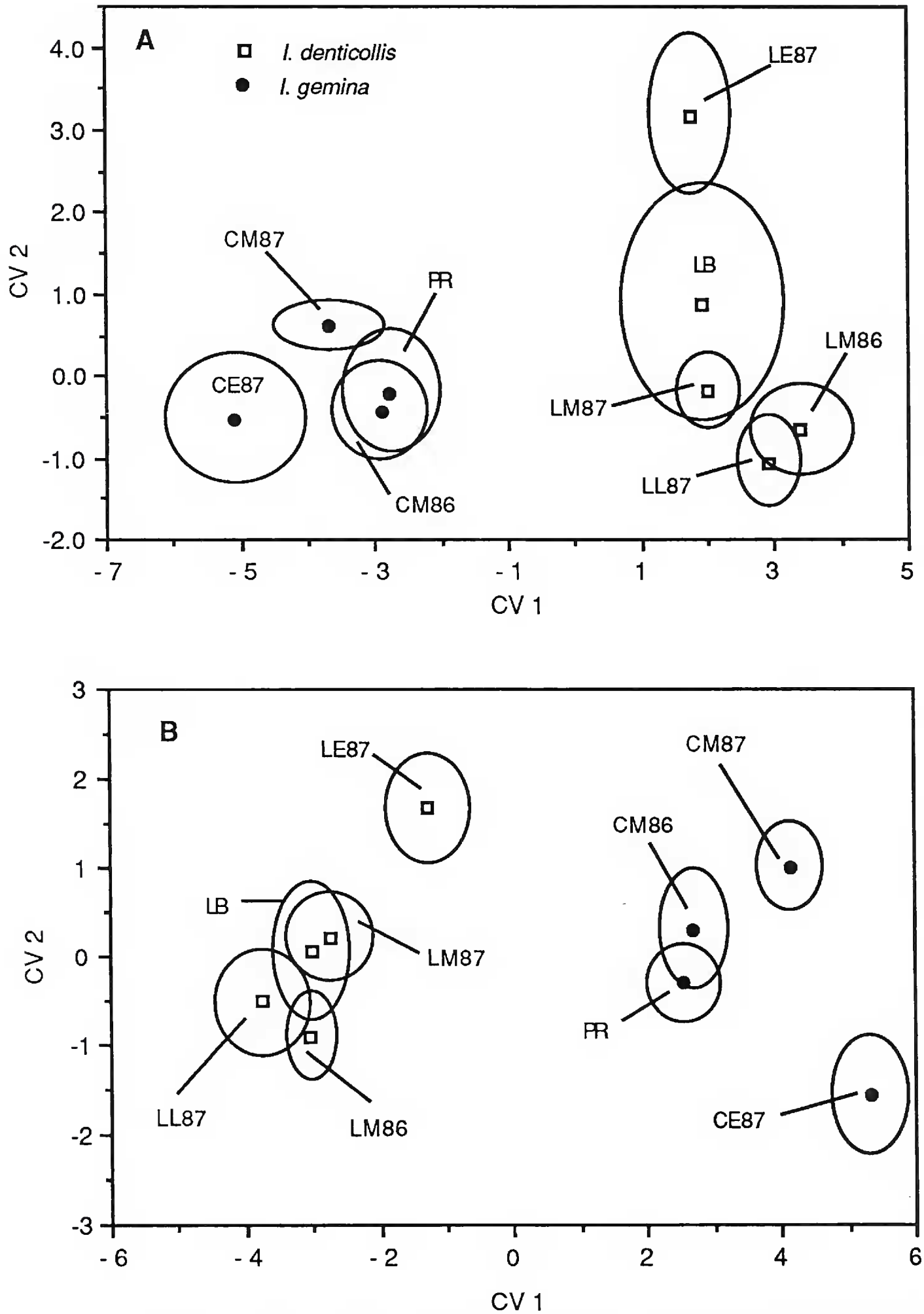


Figure 4. Sample centroids and 95% confidence ellipses from the t distribution of canonical variate scores of CV1 and CV2. A. Females. B. Males. Sample abbreviations as in Table 1.



*ticollis* Los Banos populations suggests that the Coyote Point and Livermore populations are not atypical *I. gemina* and *I. denticollis* populations. Since both the Point Reyes and Los Banos samples overlap considerably with the Coyote Point and Livermore samples, respectively, on the first and second canonical variates (Figs. 4A, 4B), it is likely that the variation within the Coyote Point and Livermore populations is fairly representative of *I. gemina* and *I. denticollis*. It is difficult, however, to assess the full amount of interpopulational variation among the *I. gemina* Coyote Point and Point Reyes and *I. denticollis* Livermore and Los Banos populations because the Point Reyes and Los Banos populations were sampled only once.

#### DISCUSSION

Canonical analysis of discriminance and classification of individuals reveal that *I. denticollis* and *I. gemina* are phenetically distinct despite the presence of strong seasonal variation within populations of both species. By sampling the *I. gemina* Coyote Point and *I. denticollis* Livermore populations repeatedly throughout the flight season, we have been able to thoroughly characterize, by morphometric means, the variation inherent in each population. This evidence indicates that the morphometric characters used in this study can be used to potentially diagnose individuals from the hybrid zone (Leong 1989). Therefore, for females, thorax length and head width may be more useful species discriminators than the structure of the prothorax, which is difficult to assess in the hybrid zone. Similarly, for males, head width and number of wing crossveins may be more useful species discriminators than the structure of the abdominal appendages. In addition, these morphometric characters have the advantage over traditional genitalic characters of being easily quantifiable, whereas differences in prothoracic or abdominal appendage structure are not.

The large amount of seasonal variation within the *I. gemina* Coyote Point and *I. denticollis* Livermore populations suggests that most of the morphometric variation in these populations is environmentally induced. The range of morphometric responses to environmental conditions seems to be limited, however, because both species are phenetically distinct. Variation in proportional head width is the predominant seasonal difference in both species and because it is correlated with overall body size, *I. gemina* and *I. denticollis* individuals also show a decrease in body size with later emergence. Similar patterns of seasonal size decrease commonly occur in natural populations of odonates (Dumont & Dumont 1969, Banks & Thompson 1985, Harvey & Corbet 1985, Van Buskirk 1987a, Baker 1989), including *I. gemina* (A. Balmy, unpublished data).

In addition to changes in body size, our data show that more subtle patterns of seasonal variation occur in *I. gemina* and *I. denticollis* as indicated by differences on the second axis (Figs. 4A, 4B). Each species and each sex within each species display separate patterns of seasonal variation. This implies that phenotypic responses to environmental variation differ between the sexes as well as between the two species. However, because random outliers are particularly prone to confound the interpretation of patterns that account for a small portion of the total variance, it is possible that variation on the second axis may reflect some random noise.

The seasonal decrease in body size of later emerging adult *I. gemina* and *I.*

*denticollis* most likely reflects the differing environmental conditions experienced by individuals during larval development. The body size of adults does not change after emergence and mortality has been found to be random with respect to size (Van Buskirk 1987a; A. Balmy, unpublished data; J. E. Hafernik, Jr., unpublished data). It is, therefore, unlikely that the seasonal size differences found in adults are an artifact of some type of size-related selection on adults. Consequently, the seasonal differences in adult size must actually represent seasonal differences in larval size in the *I. gemina* Coyote Point and *I. denticollis* Livermore populations.

Several factors may account for seasonal differences in larval size. Experimental studies of larval odonates have demonstrated the separate effects that temperature, photoperiod and food availability have on the rate of larval development and larval size (Lutz 1968, 1974a, b; Thompson 1978; Lawton et al. 1980; Harvey & Corbet 1985). Other studies have concluded that larval density affects larval size through interference competition which reduces larval feeding rates (Johnson et al. 1984, Pierce et al. 1985, Van Buskirk 1987b). Baker (1989), however, found no evidence to support this conclusion. Unfortunately, little is known about how the synergistic effect of all these factors affect larval, and thus adult size in natural populations. Harvey & Corbet (1985) suggest that overwintering in the final instar somehow causes an increase in larval size because this cohort subsequently gives rise to the larger early emerging adults. Perhaps environmental conditions experienced by the final instar most heavily influence adult size.

#### ACKNOWLEDGMENT

We thank S. C. Williams, V. T. Parker, R. W. Garrison and P. S. Ward for their helpful comments. Valuable discussions were provided by A. Balmy and D. Herlocker. This research was supported in part by a Sigma Xi Grant-in-Aid of Research.

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*Received 10 October 1991; accepted 15 January 1992.*